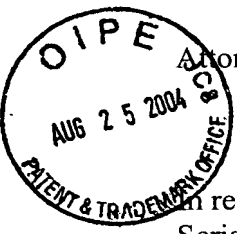


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Attorney Docket No. 9052.53

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re: Gordon Rex Paterson Dougal
Serial No.: 09/529,210
Filed: July 24, 2000
For: ELECTROMAGNETIC RADIATION THERAPY

Group Art Unit: 3739
Examiner: Henry M. Johnson, III.
Confirmation No.: 1793

Date: August 23, 2004

Mail Stop Appeal Brief-Patents
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

**TRANSMITTAL OF APPEAL BRIEF
(PATENT APPLICATION--37 C.F.R. § 1.192)**

1. Transmitted herewith, in triplicate, is the APPEAL BRIEF for the above-identified application, pursuant to the Notice of Appeal filed on June 25, 2004.
2. This application is filed on behalf of
☐ a small entity.
3. Pursuant to 37 C.F.R. § 1.17(c), the fee for filing the Appeal Brief is:

<input type="checkbox"/>	small entity	\$165.00
<input checked="" type="checkbox"/>	other than small entity	\$330.00

Appeal Brief fee due \$ 330.00

☒ Any additional fee or refund may be charged to Deposit Account 50-0220.

Respectfully submitted,

Laura M. Kelley
Registration No. 48,441

Myers Bigel Sibley & Sajovec, P.A.
P. O. Box 37428
Raleigh, North Carolina 27627
Telephone: (919) 854-1400
Facsimile: (919) 854-1401
Customer No. 20792

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Carey Gregory

Attorney Docket No. 9052.53

PATENT

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APPELLANT'S BRIEF ON APPEAL UNDER 37 C.F.R. § 1.192

Sir:

This Appeal Brief is filed pursuant to the "Notice of Appeal to the Board of Patent Appeals and Interferences" filed on June 25, 2004.

REAL PARTY IN INTEREST

The real party in interest is Virulite Limited, of Darlington, Great Britain, the assignee of this application pursuant to the Assignment from the inventor recorded at the U.S. Patent and Trademark Office on July 24, 2000 on reel number 010972 and frame number 0593.

RELATED APPEALS AND INTERFERENCES

None.

STATUS OF CLAIMS

Claims 1 and 5-26 are pending in the present application as of the filing date of this Brief. Appellant appeals the final rejection of Claims 1 and 5-26 by the Office Action dated March 25, 2004. A listing of pending Claims 1 and 5-26 is attached hereto as **Appendix A**.

STATUS OF AMENDMENTS

The following amendments have been filed in this application: (a) a Preliminary Amendment was filed on April 7, 2000; (b) an Amendment was filed on May 30, 2003; (c) an

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Amendment after Final was filed on August 27, 2002 and was entered upon the filing of a Request for Continued Examination on October 2, 2002; (d) an Amendment and Declaration of Dr. Gordon Rex Paterson Dougal under 37 C.F.R. § 1.132 was filed April 29, 2003; (e) an Amendment after Final was filed September 29, 2003; and (f) an Amendment was filed February 5, 2004. **Appendix A** includes all of the above amendments, all of which were entered in the application.

SUMMARY OF THE INVENTION

The patent application relates to an electromagnetic radiation therapy system that includes an electromagnetic radiation emitter configured to emit divergent electromagnetic radiation at a wavelength centered at 1072 nm or at a wavelength centered at 1268 nm so as to coincide with peak transmissions of a water molecule. The total bandwidth of the light is restricted so as to not exceed the bandpass filter effect characterized by the transmission spectrum of the water molecule between 980 nm and 1300 nm. The system is capable of producing, at the site being treated, a radiation intensity of at least 50 μ Watts/cm² and up to 2 Watts/cm².

As discussed in the Specification on page 2, lines 19-23, a water molecule that has a range of electromagnetic radiation wavelengths passed through it will produce several transmission peaks. A graph illustrating the peak transmissions through a water molecule is attached hereto as **Appendix B** and was submitted with the Amendment dated August 27, 2002 (previously labeled Appendix D). The transmission peaks at 1072 nm and 1268 nm are associated with preferred therapeutic wavelength ranges recited in Claim 1. It is believed that the therapeutic mechanism may be related to water and/or cell membranes. *See* Specification, page 3, lines 11-14.

The experimental results set forth in the Specification and in the articles attached hereto as **Appendix C**, which was submitted with the Amendment dated April 29, 2003 (previously labeled Exhibit A and Exhibit B), demonstrate the effectiveness of treatment at the claimed wavelengths. For example, in a double blind control trial, the average time for a patient with a herpes cold sore to be treated with 660 nm radiation was 7.5 days. The average time for a patient to be treated with 1072 nm radiation was 3 days. *See* Specification, page

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13, lines 10-15. The articles submitted as **Appendix C** reports that a single, five minute light treatment at 1072 nm reduced cold sore healing time by 4 days as compared with acyclovir applied five times daily.

ISSUES

1. Do the pending claims satisfy the provisions of 35 U.S.C. § 102(e) based on U.S. Patent No. 6,063,108 to Salansky et al. ("Salansky")?

GROUPING OF CLAIMS

For purposes of this Appeal, pending Claims 1 and 5-26 can be grouped together and stand or fall together.

ARGUMENT

The Rejection under § 102(e) should be withdrawn

A. Introduction

Appellant respectfully traverses the rejection and submits that the cited references do not disclose all of the recitations of the claims as required in a rejection under 35 U.S.C. § 102. In particular, the cited references do not disclose the wavelengths centered at 1072 nm or at 1268 nm as recited in Claim 1 with sufficient specificity.

Anticipation under § 102 requires that each and every element of the claim be found in a single prior art reference. *W. L. Gore & Associates Inc. v. Garlock, Inc.*, 721 F.2d 1540, 1554, 220 U.S.P.Q. 303, 313 (Fed. Cir. 1983). That is, all material elements of a claim must be found in one prior art source. *In re Marshall*, 198 U.S.P.Q. 344 (C.C.P.A 1978).

"Anticipation under 35 U.S.C. § 102 requires the disclosure in a single piece of prior art of each and every limitation of a claimed invention." *Apple Computer Inc. v. Articulate Systems Inc.* 57 U.S.P.Q.2d 1057, 1061 (Fed. Cir. 2000). A finding of anticipation further requires that there must be no difference between the claimed invention and the disclosure of the cited reference as viewed by one of ordinary skill in the art. *See Scripps Clinic & Research Foundation v. Genentech Inc.*, 927 F.2d 1565, 1576, 18 U.S.P.Q.2d 1001, 1010 (Fed. Cir. 1991).

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The Federal Circuit has noted that, in order to anticipate a claim, "the reference must describe the applicant's claimed invention sufficiently to have placed a person of ordinary skill in the field of the invention in possession of it." *Glaverbel Société Anonyme v. Northlake Marketing & Supply Inc.*, 33 U.S.P.Q.2d 1496, 1498 (Fed. Cir. 1995) (quoting *In re Spada*, 15 U.S.P.Q.2d 1655, 1657 (Fed. Cir. 1990)). Although a claim to a genus cannot be allowed if the prior art discloses a species falling within the claimed genus, a genus does not always anticipate a claim to a species within the genus. See M.P.E.P. § 2131.02 (citing *In re Slayter*, 276 F.2d 408, 411, 125 U.S.P.Q. 345, 347 (CCPA 1960) and *In re Gosteli*, 872 F.2d 1008, 10 U.S.P.Q.2d 1614 (Fed. Cir. 1989)). For example, if the prior art teaches a range within, overlapping, or touching the claimed range, the claimed range is anticipated only if the prior art range discloses the claimed range with "sufficient specificity." See M.P.E.P. § 2131.03 (II). The question of whether a range is disclosed with sufficient specificity is similar to that of "clearly envisioning" a species from a generic teaching. See *Id.* and M.P.E.P. § 2131.02.

The MPEP at § 2131.03 specifically addresses anticipation of a sub-genus or sub-range by a reference disclosing a broader genus or range. In particular, MPEP § 2131.03 states (emphasis added):

When the prior art discloses a range which touches, overlaps or is within the claimed range, but no specific examples falling within the claimed range are disclosed, a case by case determination must be made as to anticipation. In order to anticipate the claims the claimed subject matter must be disclosed in the reference with "sufficient specificity" to constitute an anticipation under the statute. What constitutes a "sufficient specificity" is fact dependent. If the claims are directed to a narrow range, the reference teaches a broad range, and there is evidence of unexpected results within the claimed narrow range, depending on the other facts of the case, it may be reasonable to conclude that the narrow range is not disclosed with "sufficient specificity" to constitute an anticipation of the claims. The unexpected results may also render the claims unobvious.

The Federal Circuit has addressed cases in which the claims at issue were directed to a narrow sub-range or species and a reference proposed a broad genus or range. In *Corning*

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Glass Works v. Sumitomo Electric U.S.A., Inc., 9 U.S.P.Q.2d 1962, 868 F.2d 1251, 1262 (Fed. Cir. 1989), the Federal Circuit rejected the argument that a claim in a prior art reference to a genus would inherently disclose all species. In *Ultradent Products Inc. v. Life-Like Cosmetics Inc.*, 44 U.S.P.Q.2d 1336 (Fed. Cir. 1997), the Federal Circuit held that, even if tests conducted during litigation confirmed that compositions containing carboxypolymethylene in certain concentrations provide levels of viscosity and stickiness required by the claims, the evidence did not show that the prior patent would describe the tested combinations, or other combinations meeting limitations of the claims, from among many possible candidates within the reference. *Id.* at 1342. The Federal Circuit has also upheld a finding of non-anticipation when ranges extrapolated by the patentee from the prior art patent during prosecution were "so broad as to be meaningless to one skilled in the art." See *Minnesota Mining and Manufacturing Co. v. Johnson & Johnson Orthopaedics, Inc.*, 24 U.S.P.Q.2d 1321 (Fed. Cir. 1989). In finding that the claims at issue were not anticipated, the Federal Circuit noted that "although Garwood's specific claims are subsumed in Straube's generalized disclosure of knit fiberglass as a substrate, this is not literal identity." *Id.* (emphasis added).

As discussed in more detail below, Appellant submits that the present rejections should be reversed because the prior art does not disclose with sufficient specificity an electromagnetic radiation emitter configured to emit divergent electromagnetic radiation at a wavelength centered at 1072 nm or at a wavelength centered at 1268 nm so as to coincide with peak transmissions of a water molecule.

B. Salansky does not anticipate independent Claim 1

Claims 1, 6-12 and 15-24 stand rejected under 35 U.S.C. § 102(e) as being anticipated by Salansky. Claim 1 recites as follows:

An electromagnetic radiation therapy system comprising an electromagnetic radiation emitter configured to emit divergent electromagnetic radiation at a wavelength centered at 1072nm or at a wavelength centered at 1268nm so as to coincide with peak transmissions of a water molecule, the total bandwidth being restricted so as not to

exceed the bandpass filter effect characterized by the transmission spectrum of the water molecule between 980nm and 1300nm, the system being capable of producing, at the site being treated, a radiation intensity of at least 50 $\mu\text{Watts/cm}^2$ and up to 2 Watts/cm^2 .

The Final Office Action mailed March 25, 2004 (the "March 25, 2004 Final Action") takes the position on page 1, lines 3-4 that "[t]he wavelengths cited by Salansky in claim 1, include the specific wavelengths of 1072 nm and 1268 nm of the instant application." Appellant submits that Salansky merely proposes using radiation over a broad range of wavelengths and does not identify the specific wavelengths of 1072 nm and 1268 nm as recited in Claim 1 with sufficient specificity to constitute anticipation.

In particular, Salansky proposes treating biological tissue using radiation over a broad range from 400 nm to 2000 nm. See Salansky, col. 4, line 44. Salansky treats the range as homogenous and does not focus on any specific wavelengths based on the bandpass filter effect of the water molecule. Various publications submitted on February 5, 2004, abstracts of which are attached hereto as **Appendix D**, demonstrate treatments at wavelengths that range between 660 nm and 950 nm. Neither Salansky nor the materials of **Appendix D** recognize the effect of light that coincides with the peak transmissions of a water molecule, where the total bandwidth is restricted so as not to exceed the bandpass filter effect characterized by the transmission spectrum of the water molecule between 980 nm and 1300 nm. Salansky does not discuss the transmission spectrum of the water molecule between 980 nm and 1300 nm or the shoulder of the bandpass filter effect of the water molecule from 800 nm to 1140 nm, which peaks at 1072 nm. Salansky discusses several examples within the broad range of 400 nm to 2000 nm. However, none of the examples discuss divergent electromagnetic radiation at a wavelength centered at 1072 nm or 1268 nm as recited in Claim 1. Salansky proposes treating herpes simplex and acne at wavelengths between 630 nm and 700 nm in Table 2 (Salansky, col. 9). Example 9 of Salansky proposes treating a patient suffering from herpes simplex attacks on her lips with low energy photon therapy at a wavelength of 660 nm.

Salansky does not disclose the wavelengths of Claim 1 in the present application with sufficient specificity, and, therefore, does not anticipate the pending claims. Claim 1 is directed to a relatively narrow range (*i.e.*, at a wavelength centered at 1072 nm or 1268 nm).

Salansky proposes a broad range, *i.e.*, 400-2,000 nm. Salansky does not appreciate the therapeutic effects provided by wavelengths centered at 1072 nm or 1268 nm, which coincide with the peak transmissions through a water molecule. Indeed, Salansky depends on reduced bandwidth (*i.e.*, a bandwidth that should not exceed 30-40 nm (*see* col. 16, lines 39-43)) for efficacy irrespective of wavelength. Appellant submits that the broad range of 400-2,000 nm proposed by Salansky, which identifies a range of wavelengths that is 1600 nm wide, would not lead one of skill in the art to choose electromagnetic radiation centered at a wavelength of 1072 nm or 1268 nm as recited in Claim 1 from the many possible wavelength candidates proposed by Salansky. In light of the narrow range claimed, which is centered at two specific wavelengths, the range of wavelengths proposed by Salansky is so broad as to be meaningless for purposes of anticipation.

Therefore, the rejection of Claim 1 and Claims 5-26 depending therefrom should be reversed because Salansky does not disclose the narrow range of a wavelength centered at 1072 nm or 1268 nm with sufficient specificity.

Although evidence of secondary considerations, such as unexpected results, is not relevant to a rejection under § 102 (*see* M.P.E.P. § 2131.04), evidence of unexpected results may be used to rebut a rejection of a range of values under § 103 (*see* M.P.E.P. § 2144.05 (III)). The claims under appeal in the present application stand rejected based on § 102 only, and therefore, evidence of unexpected results has not been addressed in detail. However, it is noted that the data in the Specification and in **Appendix C** attached hereto and the Declaration of Dr. Gordon Rex Paterson Dougal under 37 C.F.R. § 1.132 filed April 29, 2003 demonstrate the effectiveness of the wavelengths recited in Claim 1 over the wide range of wavelengths proposed in Salansky for the treatment of herpes cold sores.

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C. Conclusion

On the entire record and in view of all the cited references, Appellant submits that Claims 1 and 5-26 are novel and non-obvious. Accordingly, it is respectfully requested that the Examiner's conclusions be reversed, and that this case be passed to issuance.

Respectfully requested,



Laura M. Kelley
Registration No. 48,441

Myers Bigel Sibley & Sajovec, P.A.
P. O. Box 37428
Raleigh, North Carolina 27627
Telephone: (919) 854-1400
Facsimile: (919) 854-1401
Customer No. 20792

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Carey Gregory

APPENDIX A

1. An electromagnetic radiation therapy system comprising an electromagnetic radiation emitter configured to emit divergent electromagnetic radiation at a wavelength centered at 1072nm or at a wavelength centered at 1268nm so as to coincide with peak transmissions of a water molecule, the total bandwidth being restricted so as not to exceed the bandpass filter effect characterized by the transmission spectrum of the water molecule between 980nm and 1300nm, the system being capable of producing, at the site being treated, a radiation intensity of at least 50 $\mu\text{Watts}/\text{cm}^2$ and up to 2 Watts/cm^2 .

5. An electromagnetic radiation therapy system according to Claim 1 wherein the half angle divergence of the electromagnetic radiation is in the range 15° to 45° .

6. An electromagnetic radiation therapy system according to Claim 1 wherein the electromagnetic radiation is continuous or pulsed.

7. An electromagnetic radiation therapy system according to Claim 1 wherein the electromagnetic radiation is continuous, and the intensity is at least 50 $\mu\text{Watts}/\text{cm}^2$ for treatment of eyes and mucous membranes and up to 2 Watts/cm^2 .

8. An electromagnetic radiation therapy system according to Claim 1 wherein the electromagnetic radiation is continuous, and the intensity is at least 500 $\mu\text{Watts}/\text{cm}^2$ for treatment of skin and up to 2 Watts/cm^2 .

9. An electromagnetic radiation therapy system according to Claim 1 wherein the electromagnetic radiation is pulsed, and the intensity is at least 50 $\mu\text{Watts}/\text{cm}^2$ peak power for treatment of eyes and mucous membranes and the average power is up to 2 Watts/cm^2 .

10. An electromagnetic radiation therapy system according to Claim 1 wherein the electromagnetic radiation is pulsed, and the intensity is at least $500 \mu\text{Watts}/\text{cm}^2$ peak power for treatment of skin and the average power is up to $2 \text{ Watts}/\text{cm}^2$.

11. An electromagnetic radiation therapy system according to Claim 1 wherein the electromagnetic radiation is pulsed, and the average power of the pulsed electromagnetic radiation intensity is in the region of $50\text{-}100 \mu\text{Watts}/\text{cm}^2$.

12. An electromagnetic radiation therapy system according to Claim 1 wherein the electromagnetic radiation is pulsed, and the pulsed electromagnetic radiation is applied for pulse duration periods of at least $10\text{-}15 \mu\text{seconds}$.

13. An electromagnetic radiation therapy system according to Claim 1 wherein the electromagnetic radiation is pulsed, and the pulsed electromagnetic radiation is applied at a frequency/repetition rate in the range $480\text{-}800 \text{ Hz}$.

14. An electromagnetic radiation therapy system according to Claim 13 wherein the frequency/repetition rate is at, or about, 600 Hz .

15. An electromagnetic radiation therapy system according to Claim 1 wherein the electromagnetic radiation is pulsed, and the pulsed electromagnetic radiation is applied to the affected area for at least 30 seconds and up to 15 minutes.

16. An electromagnetic radiation therapy system according to Claim 1 wherein the electromagnetic radiation therapy system also includes means for reducing the amount of ambient radiation which impinges on the site of treatment.

17. An electromagnetic radiation therapy system according to Claim 16 wherein the means for excluding ambient radiation excludes radiation below $400\text{-}500 \text{ nm}$.

18. An electromagnetic radiation therapy system according to Claim 1 further including means for fixing the intensity of the radiation within a predetermined range.

19. An electromagnetic radiation therapy system according to Claim 1 wherein radiation output is monitored with a visible display indicating correct function of the device both for intensity and wavelength.

20. An electromagnetic radiation therapy system according to Claim 1 further including means for controlling the duration of the application of the radiation.

21. An electromagnetic radiation therapy system according to Claim 1 wherein the radiation producing means are solid state light emitting devices.

22. An electromagnetic radiation therapy system according to Claim 21 wherein the solid state light emitting devices are solid state light emitting diodes or gas discharge devices or a laser diode device.

23. An electromagnetic radiation therapy system according to Claim 21 wherein radiation from said solid state light emitting devices is electrically operated or delivered to an applicator via a fibre-optic delivery system.

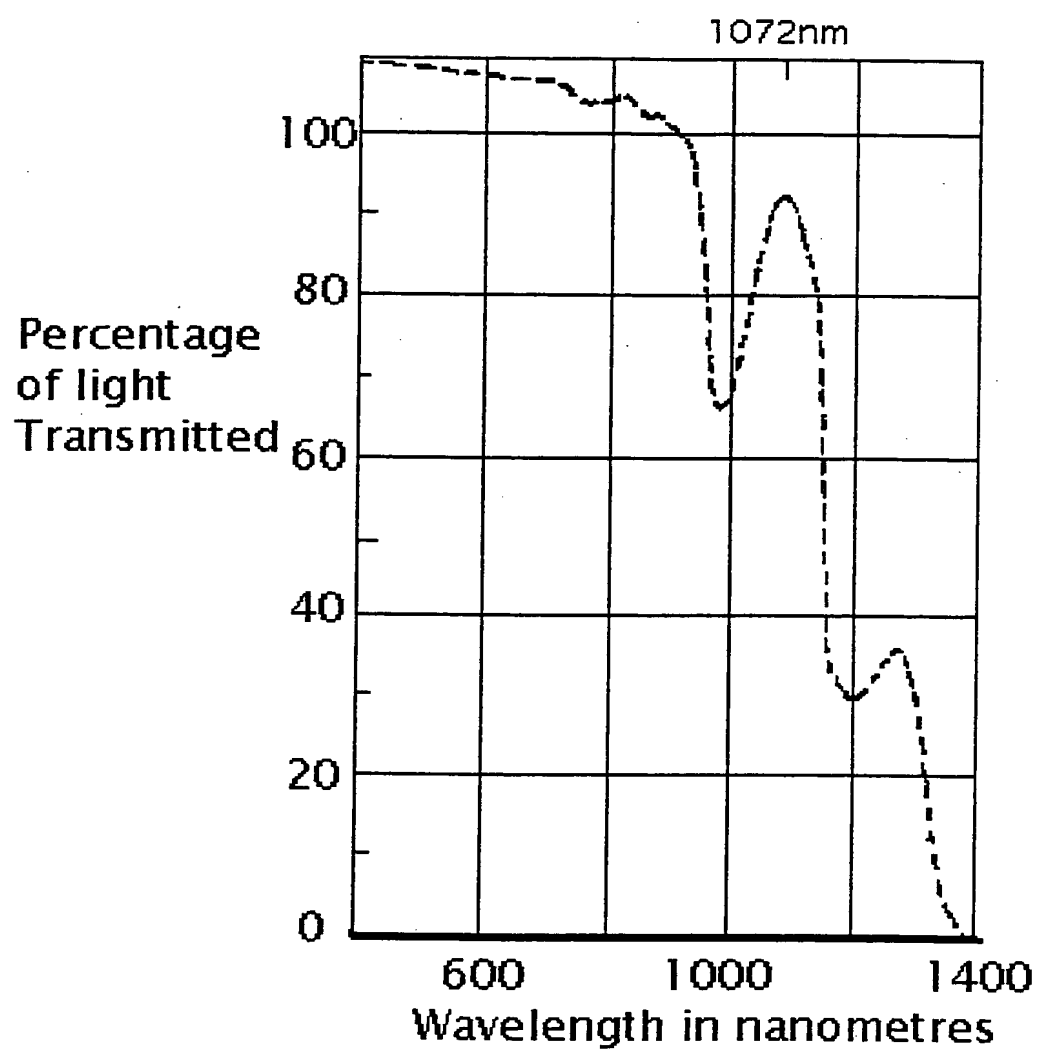
24. An electromagnetic radiation therapy system according to Claim 21 wherein the means for emitting includes a PN junction arranged to emit radiation with a wavelength centring at, or about, 1072nm or at, or about, 1268 nm.

25. An electromagnetic radiation therapy system according to Claim 24 comprising a single light diode assembly including a plurality of orientated junctions.

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26. An electromagnetic radiation therapy system according to Claim 22 wherein the gas discharge device may include a mixture of gases which will give an output at the desired wavelength centered at 1072 nm or 1268 nm.

APPENDIX B



Clinical and Experimental Dermatology

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A pilot study of treatment of herpes labialis with 1072 nm narrow waveband light

G. Dougal and P. Kelly

Occupational Health Department, North Tees Hospital, Stockton-on-Tees, UK

Appendix C

Summary

A randomized prospective double-blind study was performed to compare the efficacy of a single 5 min 1072 nm narrow waveband light application against topical aciclovir applied five times daily in the treatment of herpes labialis. Treatment was initiated within 36 h of the onset of symptoms and the end point was defined as the day that the crust was discarded leaving an uninterrupted underlying skin at the site of the cold sore. The results demonstrated that a single 5 min light treatment significantly reduced cold sore healing time by 4 days; 1072 nm light healed cold sores in 4.3 ± 1.8 days (mean \pm SD) as compared with aciclovir applied five times daily, 8.5 ± 3.0 days ($P < 0.0001$).

Background

Although infrared light is recognized as a treatment of musculo-skeletal disorders and indolent wounds, the evidence that it has therapeutic effect remains anecdotal. Indeed, until very recently the results of clinical trials exploring proposed therapeutic effects of infrared light had not been documented with meaningful statistical significance.¹⁻⁶ In 1999, however, Schindl and Neumann demonstrated that low intensity laser therapy is an effective nonthermic treatment for recurrent herpes simplex infection.⁷

In the laboratory various photobiological effects of infrared light have been explored, albeit dictated by the random commercial availability of predominately laser light sources.⁸⁻¹³ These well-documented experiments have demonstrated unequivocally that selected wavelengths of infrared light have nonthermal photobiological effect. We were unable to find any evidence to suggest that these laboratory results had been correlated with the known anecdotal clinical therapeutic effects of infrared light.

We hypothesized that within the infrared spectrum

there might be one or more narrow wavebands of light with therapeutic photobiological effect. As long ago as 1981 Anderson and Parrish¹⁴ introduced the possibility of treating large tissue volumes with certain long wavelength photosensitisers within the optical window of skin, between 600 and 1300 nm. We reasoned that tissue penetration would be influenced by light transmitted by water, which represents the major component of the human body. Examination of the transmission spectrum of the water molecule demonstrated a peak transmission of light with a wavelength of 1072 nm (Fig. 1).

For this study we decided to use a narrow waveband of light centred at 1072 nm using quantities of light which would not have thermal effect. (Terms: 1072 NWBL = light with a centre wavelength of 1072 nm and a bandwidth of ± 20 nm).

Cold sores appeared to be the obvious choice when searching for a clinical model to observe the effects of light therapy. They are known to be activated by exposure to ultraviolet light¹⁵ and recurrence rates are known to be reduced by exposure to low intensity laser therapy. Approximately 20% of the world's population suffer from cold sores, providing within the community a potentially large number of patient volunteers to be recruited into clinical trials.

Correspondence: G. Dougal, 6 Whitewicks, Durham DH1 4HW.
Tel: +44 778 2567315. Fax +44 191 3842424.

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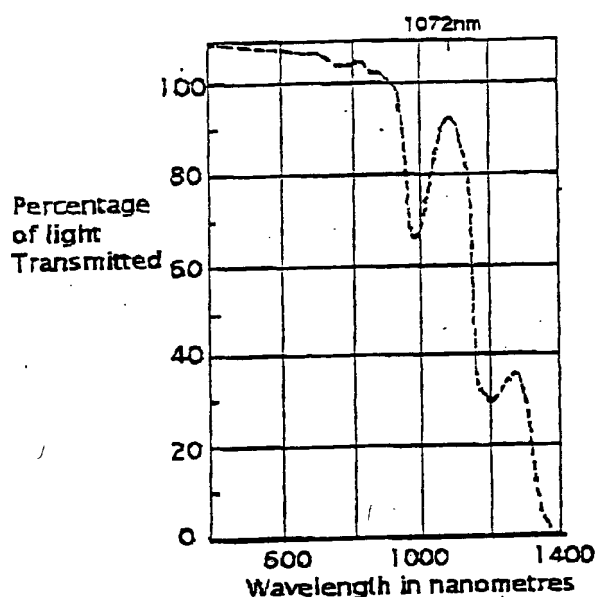


Figure 1 Light transmission spectrum of water between wavelengths 400 nm and 1400 nm.

Patients and methods

Protocol

Patient volunteers were recruited by advertisement within the local press after ethics permission had been obtained. Informed consent was obtained from all volunteer patients.

The interventions compared were a single 5 min treatment of 1072 NWBL vs. five times daily topical aciclovir applied until the cold sore was reported to be cured. Despite medical publications^{16,17} to the contrary, topical aciclovir appears to be accepted by the general population as treatment of choice for cold sores. The duration of the cold sore must have been 36 h or less in all volunteers. The time of onset of the cold sore was defined as either the time of onset of symptoms or first appearance of the lesion, whichever was the soonest.

The initial parameters measured were cold sore size and the duration of the cold sore prior to intervention. Cold sore size was documented by photograph and the largest diameter measured by ruler.

The key outcome variable was the time at which the cold sore was cured, defined as the time when the crust had fallen off the cold sore leaving uninterrupted skin at the site. This was verified by the patient on a written

response questionnaire and validated visually by an independent observer blind to treatment.

The possibility that using a light treatment device would have a placebo effect was explored by subdividing those patients receiving topical aciclovir into two groups: group 1 receiving only aciclovir and group 2 receiving aciclovir and placebo light. In a similar way any therapeutic effect of the placebo cream, advantageous or otherwise, was explored by treating half of the 1072 NWBL group with placebo cream.

The protocol was approved by North Tees General Hospital Ethics Committee.

Randomization method

The individuals were allocated to receive one of four treatments without restriction according to a standard computer-generated randomization table. Each treatment type was allocated an alphabetical letter which was assigned randomly to the patient number. Patient numbers were allocated sequentially. Each treatment arm was housed in a separate lettered container.

It was deemed unethical to withhold treatment from subjects, hence there is not a placebo control group in the study (i.e. either placebo light only or placebo cream only).

The 4 groups ran concurrently and were delivered the following treatments: group 1, topical aciclovir five times daily; group 2, topical aciclovir five times daily plus placebo light once for 5 min; group 3, 1072 NWBL once for 5 min; group 4, 1072 NWBL once for 5 min plus placebo cream five times daily.

Method of masking

The pharmaceutical creams were labelled with the patient number alone in Hartlepool General Hospital pharmacy. The pots in which the creams were dispensed were identical in external appearance and the quantity, consistency, colour and odour of the placebo cream appeared identical to topical aciclovir.

As the light is invisible to the human eye the external appearance of the light applicators and their external functions were identical. There was no mechanism by which either the patient or the researchers could discriminate between groups 2 and 4, and a separate staff member who independently followed-up the patients was blind to all four treatment arms. The code was located at Hartlepool General Hospital in a sealed envelope and was broken only at the conclusion of the trial after data analysis. The code was inaccessible to both the individuals carrying out the intervention and the outcome assessor who visually confirmed that the cold sore was healed. The

Table 1 Comparison of the four treatment groups

	Patients treated between 18 and 36 h of onset of cold sore, % of total, (n)	Mean cold sore diameter [mm \pm SD (n)]	Nurse observed cold sore cured [mean days \pm SD (n)]	Patient reported cold sore cured [mean days \pm SD (n)]
Light @1072 nm single 5 min application	8, 73% (11)	2.5 \pm 1.1 (11)	7.0 \pm 3.2 (11)	4.6 \pm 2.2 (11)
Light @1072 nm single application plus placebo cream five times daily	10, 71% (14)	3.3 \pm 1.9 (14)	8.7 \pm 3.9 (9)	4.0 \pm 1.4 (14)
Aciclovir cream five times daily	10, 71% (14)	3.3 \pm 1.7 (14)	12.1 \pm 4.3 (12)	8.8 \pm 3.5 (14)
Aciclovir cream five times daily plus a single application of placebo light	11, 92% (12)	2.7 \pm 1.0 (12)	10.6 \pm 4.5 (12)	8.1 \pm 2.5 (12)
P value	Lowest P = 0.32	P = 0.45	P = 0.025	P < 0.0001

data was analysed independently by The University of Teesside Medical Research Department prior to decoding.

Apparatus

The light source used a multimode laser diode array centred at 1072 nm with a bandwidth of ± 20 nm. The optical power was maintained between 5 and 10 mW/cm² peak power at the skin surface, switched at 600 Hz with a 20% duty cycle. Internal monitoring of the light output ensured that treatment parameters remained constant. The treatment area was constant at 6 cm². The device, a class I laser product, operated from a 5 V double insulated supply with less than 20 μ A earth leakage and contained an internal timer which facilitated a constant treatment time of 5 mins.

Statistics

Conventional one-way analysis of variance was used to compare cold sore size and days to heal among the four

treatment groups. The two-sample t-test was used to compare the pooled aciclovir and 1072 NWBL groups.

For the proportion of patients treated between 18 and 36 h of onset, the four treatment groups were compared by applying the Fisher exact test to each pair of groups. This incurred three tests rather than six because the numbers from two of the groups were identical (Table 1). A single Fisher exact test was used to compare the pooled aciclovir and 1072 NWBL groups.

The statistical analysis was carried out using Minitab version 12.

Results

The data was analysed on an intention-to-treat basis.

Sixty volunteers were recruited into the trial. Eight patients were lost to follow-up and one patient with acne was excluded (Fig. 2). In the 1072 NWBL treatment group, 18 females and seven males were recruited and in the aciclovir treatment group, 22 females and four males were recruited. The mean age of

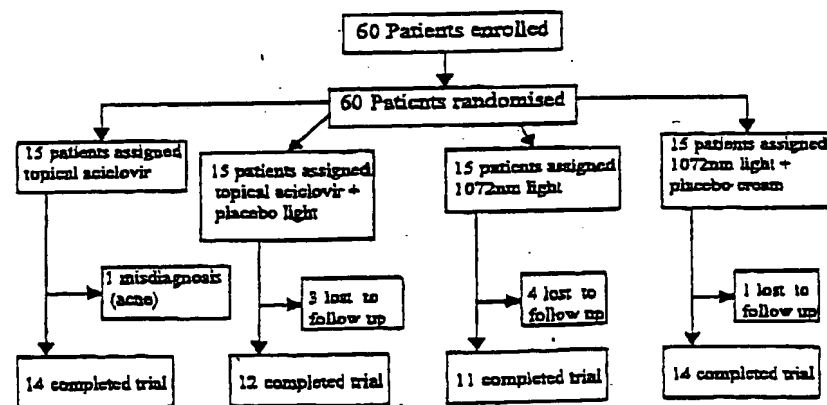


Figure 2 Trial profile.

Table 2 Pooled groups, active light vs. topical aciclovir

	Patients treated between 18 and 36 h of onset of cold sore, % of total, (n)	Mean cold sore diameter [mm \pm SD (n)]	Nurse observed cold sore cured [mean days \pm SD (n)]	Patient reported cold sore cured [mean days \pm SD (n)]
Active light, single 5 min treatment	18, 78% (25)	2.91 \pm 1.53 (25)	7.8 \pm 3.5 (20)	4.3 \pm 1.8 (25)
Topical aciclovir five times daily	21, 87% (26)	3.0 \pm 1.23 (26)	11.3 \pm 4.3 (24)	8.5 \pm 3.0 (26)
P value	P = 0.46	P = 0.82	P = 0.005	P < 0.0001
95% confidence interval of the difference			1.1–6.0	2.6–5.7

the 1072 NWBL treatment group was 41.8 years (range, 24–66 years) and the mean age of the aciclovir treated group was 40.3 years (range, 23–54 years).

Table 1 column 1 shows that the time interval between onset of symptoms and initiating treatment (less than 18 h or 18–36 h) was not significant between the groups ($P = 0.32$). Column 2 shows that the baseline parameter of cold sore size at the onset of treatment was not significantly different between the groups ($P = 0.45$).

Self reported time to cure

Table 1 column 4 shows the self-reported time to cure for each treatment arm. The two 1072 NWBL groups are reported as cured in about half the time than the two aciclovir groups, approximately 4 days vs. 8 days ($P < 0.0001$).

Table 2 is a comparison of the pooled 1072 NWBL groups vs. the pooled aciclovir groups. The results in

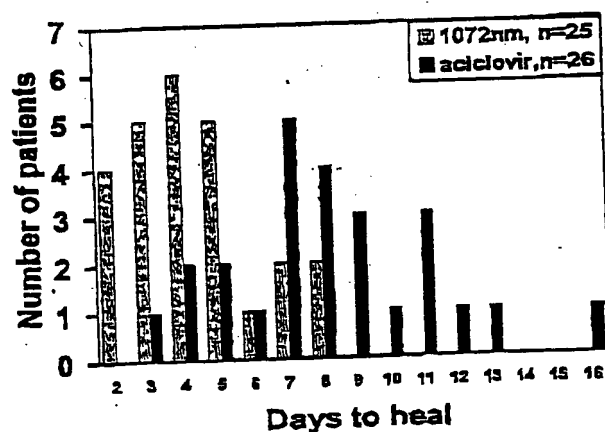


Figure 3 1072 nm light vs. aciclovir in the treatment of herpes labialis.

column 4 compare the self-reported time to cure of the pooled groups and are also represented as a histogram in Fig. 3. The 1072 NWBL group is reported as healed in 4.3 days vs. 8.5 days in the aciclovir group ($P < 0.0001$).

Once again there is no significant difference in the baseline parameters of cold sore size and the time of onset of treatment between the 1072 NWBL and aciclovir treated groups.

Nurse observed cold sore cured

The time at which the healed cold sore was available to be observed by the outcome assessor nurse was subject to a variable delay (Tables 1 and 2, column 3) affected by communication, transport and convenience.

However, the delays should have balanced out between the groups and there was no reason to suspect that any one group was seen sooner or later than the others. The results show a very similar pattern to those described for the self-reported time to cure, aciclovir 11.3 \pm 4.3 days, 1072 nm light 7.8 \pm 3.5 days, albeit with reduced statistical significance ($P = 0.005$).

Discussion

This study demonstrates statistically significant evidence from a randomized controlled trial that patients treated with 1072 NWBL within 36 h of onset of herpes labialis reported that their cold sores healed in half the time (4 days) compared with patients treated with conventional medication (8 days) in the form of aciclovir cream. To our knowledge this is the first time that a narrow waveband of light has been demonstrated to cause shortened cold sore healing time with a meaningful statistical significance. The difference in healing time was not influenced by the size of the cold sore. For both the 1072 NWBL and aciclovir treatment groups there was a placebo control for comparison and

all outcomes were recorded blind to the treatment received by the subject.

Previous research has shown that similar types of phototherapy using athermic quantities of low energy red or near infrared monochromatic light have been used for the acceleration of wound healing^{18, 19} and in pain therapy.^{20, 21} In addition it has been reported that this type of phototherapy might have an effect on several immunological reactions^{22, 23} and is an effective treatment in preventing recurrent herpes simplex infection. *In vitro* investigations have not found any evidence to suggest that infrared irradiation inactivates the herpes simplex virus within infected cells.²⁴

The mechanism by which infrared light has photobiological effect at molecular level, either demonstrated clinically, or by laboratory experiment, remains unexplained. We might imagine a hypothesis whereby cell membranes are the main beneficiary of light energy within the vicinity of 1072 nm. A more efficient cell membrane would increase resistance of the cell to virus entry, exposing the virus to an enhanced local immune response. Wound healing and repair might equally be enhanced.

In the light of our findings we would like to think that an increased awareness of the potential of infrared light to treat disease will stimulate further studies. Co-ordinated research would enable a map to be plotted of the therapeutic potential of light across the infrared spectrum. Of particular interest might be light within the optical window of skin (600–1300 nm) which would, in theory, have potential applications in the treatment of systemic disease processes. In the meantime we think that the knowledge that 1072 NWBL has therapeutic effect deserves further study, with respect to both dermatological and systemic disease.

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660nm LEDs is ineffective in the treatment of cold sores

Summary

A single blind randomised controlled trial demonstrated that 660nm LEDs are ineffective in the treatment of cold sores.

Introduction

Over the years HeNe and semiconductor diode lasers¹ have been used with claimed therapeutic benefit in the treatment of cold sores, some have advocated the use of red light emitting diodes (LEDs) in the treatment of cold sores. A prospective single blind randomised controlled trial examines the efficacy of 660nm LEDs in the treatment of cold sores.

Patients and Methods

Protocol

Patient volunteers were recruited by advertisement within the local press. Informed consent was obtained from all volunteer patients.

Each light source produced the same optical power output of 5 - 10mw / sq cm microprocessor controlled to ensure that the light intensity remains constant.

The interventions compared were a single 5-minute treatment of 660nm light versus 5 times daily topical aciclovir applied until the cold sore was reported to be cured.

The duration of the cold sore must have been 36 hours or less in all volunteers. The time of onset of the cold sore was defined as either the time of onset of symptoms or first appearance of the lesion, whichever was the soonest.

The initial parameters measured were cold sore size and the duration of the cold sore prior to intervention. Cold sore size was documented by photograph and largest diameter.

The key outcome variable was the time at which the cold sore was cured, defined as the time when the crust had fallen off the cold sore leaving uninterrupted skin at the site. This was verified by the patient on a written response questionnaire and validated visually by an independent observer blind to treatment.

The possibility that using a light treatment device would have a placebo effect was explored by sub-dividing those patients receiving topical aciclovir into two groups: group 1 receiving only aciclovir and group 2 receiving aciclovir and placebo light. In a similar way any therapeutic effect of the placebo cream, advantageous or otherwise, was explored by treating half of the 660nm group with placebo cream.

North Tees General Hospital Ethics Committee approved the protocol:-

Randomisation Method

The individuals were allocated to receive one of four treatments without restriction according to a standard computer generated randomisation table. Each treatment type was allocated an alphabetical letter that was assigned randomly to the patient number. Patient numbers were allocated sequentially. Each treatment arm was housed in a separate lettered container.

It was deemed unethical to withhold treatment from subjects and hence there is not a placebo control group in the study (i.e. either placebo light only or placebo cream only).

The 4 groups ran concurrently and were delivered the following treatments:

Group 1 Treated with topical aciclovir 5 times daily

Group 2 Treated with topical aciclovir 5 times daily plus placebo light once for 5 minutes

Group 3 Treated with active light once for 5 minutes

Group 4 Treated with active light once for 5 minutes plus placebo cream 5 times daily.

Method of Masking

The pharmaceutical creams were labelled with the patient number alone in Hartlepool General Hospital pharmacy. The pots in which the creams were dispensed were identical in external appearance and the quantity, consistency, colour and odour of the placebo cream appeared identical to topical aciclovir.

As the light is visible to the human eye blinding to the staff treating the volunteers was not possible. The device was shielded from the volunteers and placed against the skin and then activated, thus the patients were unaware of the light emissions. The external appearance of the light applicators and their external functions were identical.

There was no mechanism by which the patient could discriminate between groups delivering aciclovir + placebo light and active light + placebo cream and a separate

staff member who independently followed up the patients was blind to all 4 treatment arms. The code was located at Hartlepool General Hospital in a sealed envelope and was only broken at the conclusion of the trial after data analysis. The code was inaccessible to both the individuals carrying out the intervention and the outcome assessor who visually confirmed that the cold sore was healed.

Apparatus

The 660nm \pm 30nm utilised a LED array, the optical power was maintained at 5-10mw/sq cm at the skin surface. Internal monitoring of the light output in all devices ensured treatment parameters remained constant. The treatment area was constant at 6 cm². The devices operated from a 5-volt double insulated supply with less than 20 micro amps earth leakage and contained an internal timer that facilitated a constant treatment time of five minutes.

Statistics

Conventional one-way analysis of variance was used to compare cold sore size and days to heal among the four treatment groups. The two-sample t-test was used to compare the pooled aciclovir and 660nm groups.

For the proportion of patients treated between 18-36 hours of onset, applying the Fisher exact test to each pair of groups compared the four treatment groups. This incurred three tests rather than six because the numbers from two of the groups were identical (Table 1). A single Fisher exact test was used to compare the pooled aciclovir and 660nm groups.

Results

The data was analysed on an intention to treat basis.

Table 1.

	Patients treated between 18 – 36 hours of onset of cold sore, percentage of total, (n)	Mean cold sore diameter mm \pm standard deviation, (n)	Nurse observed cold sore cured, mean days \pm standard deviation, (n)	Patient reported cold sore cured, mean days \pm standard deviation, (n)
Light @ 660nm single 5 min application	4 40% (10)	3.6 \pm 3.4 (10)	9.6 \pm 3.8 (10)	8.6 \pm 4.4 (10)
Light @ 660nm – single application plus placebo cream 5 times daily	3 30% (10)	2.8 \pm 1.54 (10)	9.3 \pm 3.6 (10)	8.2 \pm 3.7 (10)
Aciclovir cream 5 times daily	6 67% (9)	2.1 \pm 1.1 (9)	9.1 \pm 4.6 (9)	8.1 \pm 4.6 (9)
Aciclovir cream 5 times daily plus a single application placebo light	3 33% (10)	3.0 \pm 2.64 (10)	10.2 \pm 4.3 (9)	8.8 \pm 4.0 (10)
p-value		p=0.	p=	p

Table 2. Pooled groups, 660nm light vs aciclovir

	Patients treated between 18 – 36 hours of onset of cold sore, percentage of total, (n)	Mean cold sore diameter mm \pm standard deviation, (n)	Nurse observed cold sore cured, mean days \pm standard deviation, (n)	Patient reported cold sore cured, mean days \pm standard deviation, (n)
Active light, a single 5 minute treatment	6 30% (20)	3.2 \pm 2.6 (20)	9.4 \pm 3.6 (20)	8.4 \pm 4.2 (20)
Topical aciclovir 5 times daily	9 50% (18)	2.55 \pm 2.0 (18)	9.7 \pm 4.2 (18)	8.6 \pm 3.8 (19)
p-value	p=.	p=.	p=	p

Discussion

Whilst red light¹ has been demonstrated to reduce recurrence of cold sores when used in higher power densities 660nm light from LEDs is ineffective in reducing cold sore healing time. Aciclovir has been demonstrated to be effective in the prodromal phase only, hence by the time treatment with topical acyclovir was initiated the period during which time it would have been effective had expired^{2,3}.

Whilst numbers are small, should there have been a marginal therapeutic benefit the numbers are sufficient to demonstrate a trend in that direction, this has not occurred.

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Double-blind, placebo-controlled investigation of the effect of combined phototherapy/low intensity laser therapy upon experimental ischaemic pain in humans.

Mokhtar B, Baxter GD, Walsh DM, Bell AJ, Allen JM.

Department of Occupational Therapy and Physiotherapy, University of Ulster, Jordanstown, Northern Ireland, U.K.

BACKGROUND AND OBJECTIVE: This study assessed the putative analgesic effect of combined monochromatic light/laser irradiation at low intensity (660-950 nm; 31.9 J/cm²; pulsed at 16 or 73 Hz). **STUDY DESIGN/MATERIALS AND METHODS:** The investigation was completed under double-blind conditions using a standardised form of the submaximal effort tourniquet technique. Healthy male volunteers naive to the experimental conditions (n = 45) attended on two occasions for the purposes of pain induction, the first during which baseline data were obtained and on a second occasion during which they were randomly allocated to one of two treatments or a placebo condition. For the treatment conditions, irradiation was applied to the ipsilateral Erb's point at the parameters stated; for the placebo condition, sham "irradiation" was delivered using a dummy unit. Pain was measured using computerised visual analogue scales and McGill Pain Questionnaires (MPQ) to assess "current pain intensity" and "worst pain experienced," respectively. **RESULTS:** Analysis of variance and appropriate post hoc tests demonstrated only a weak (but significant) hypoalgesic effect compared to placebo ($P < 0.05$) in the treatment group irradiated at 16Hz for the sensory component of the MPQ; other comparisons were found to be nonsignificant. **CONCLUSIONS:** These results do not provide convincing evidence for the hypoalgesic potential of combined monochromatic light/laser irradiation, at least at the parameters used here, and thus indicate the necessity of additional work to investigate this modality further in order to assess the potential benefit, if any, of such treatment in the clinical setting.

Publication Types:

- Clinical Trial

Br J Rheumatol. 1993 Aug;32(8):740-2.

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A double-blind study of the effectiveness of low level laser treatment of rotator cuff tendinitis.

Vecchio P, Cave M, King V, Adebajo AO, Smith M, Hazleman BL.

Rheumatology Research Unit, Addenbrooke's Hospital, Cambridge.

Thirty-five patients with rotator cuff tendinitis were randomly allocated to active (CB Medico Master III 830 nm Ga As AL diode) laser or dummy laser treatment twice weekly for 8 weeks. Movement range, painful arc score, resisted movement score and responses to visual analogue scales for night pain, rest pain, movement pain and functional limitation were measured second weekly. All responses improved from baseline but there was no difference between the two groups. These results fail to demonstrate the effectiveness of laser therapy in rotator cuff tendinitis.

Publication Types:

- Clinical Trial
- Randomized Controlled Trial

PMID: 8348278 [PubMed - indexed for MEDLINE]

*Effect of a helium-neon laser on cutaneous inflammation.***Sakihama H.***Department of Dermatology, Kurume University School of Medicine, Japan.*

A Helium-Neon (He-Ne) laser with a wavelength of 632.8 nm is known to have photobiological effects and is widely used for reducing the pain of herpes zoster and accelerating wound healing, however the cellular mechanism and effect of the He-Ne laser are poorly understood. The present study was designed to examine the influence of He-Ne laser irradiation on irritant and allergic contact dermatitis of the mouse ear and on histamine release from rat peritoneal mast cells. Irradiation was applied with a He-Ne laser (12.2 J/cm²) at 1 h, 10 min, 5 min and 0 min before, and 5 min, 6 hs and 24 hs after a challenge of an irritated contact dermatitis (ICD) or allergic contact dermatitis (ACD) was made on the right ears of ICR-mice. Twenty-four hours after the challenge, the swelling of the ear was measured with a dial thickness gauge, and the anti-inflammatory effect of He-Ne laser irradiation was expressed as an ear thickness ratio (ETR). Although the laser did not decelerate the ETR from ICD, the allergic response was decelerated. Irradiation at 5 min after the challenge of contact dermatitis increased the thickness ratio. Next, the influence of the He-Ne laser on histamine release from Wistar-rat peritoneal mast cells was observed. The spontaneous histamine release was inhibited by laser irradiation, while substance P and compound 48/80-induced histamine release were not inhibited. From these results, it can be suggested that He-Ne laser irradiation has an anti-inflammatory effect on cutaneous inflammation.

Lasers Surg Med. 1984;3(4):279-84.

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Nonthermal effects of ND:YAG laser on biological functions of human skin fibroblasts in culture.

Abergel RP, Meeker CA, Dwyer RM, Lesavoy MA, Uitto J.

Previous studies have indicated that laser can selectively affect the biological functions of cells. In the present study, the role of a thermal component in laser-induced alterations in the biology of human skin fibroblasts was examined. Cells were cultured on 96-well tissue culture plates, subjected to treatment with the Nd:YAG laser (wavelength 1,064 nm), and the temperature of the medium was monitored by a microprobe connected to a telethermometer. For comparison, parallel cultures were heated to the same temperatures by tungsten-halogen lamp. The cell cultures were analyzed for collagen synthesis by incubating the cultures with [3H]proline, and the collagen production was assayed by the synthesis of nondialyzable [3H]hydroxyproline. The rate of DNA replication was also determined by measuring the uptake of [3H]thymidine. A marked decrease of collagen production and thymidine incorporation was noted in the cultures subjected to Nd:YAG laser. No such decreases were noted in cultures heated to the corresponding temperatures by tungsten-halogen lamp. The results thus indicate that the biochemical alteration caused by the Nd:YAG laser in human fibroblast functions cannot be explained on the basis of thermal effects.

Ann Plast Surg. 1983 Sep;11(3):214-22.

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Effects of the Nd:YAG laser on DNA synthesis and collagen production in human skin fibroblast cultures.

Castro DJ, Abergel RP, Meeker C, Dwyer RM, Lesavoy MA, Uitto J.

Human skin fibroblasts were subjected to treatment with a Neodymium:YAG laser at 1060 nm with varying levels of energy determined by a reproducible method of dosimetry. DNA synthesis in the cells was measured by the incorporation of [3H]thymidine, and collagen production was monitored by the synthesis of nondialyzable [3H]hydroxyproline after incubation of cells with [3H]proline. Using energy levels equal to 1.7×10^3 J/cm², a significant reduction in DNA synthesis was noted, while the cells remained viable as tested by the trypan blue exclusion test. With energy levels higher or equal to 2.3×10^3 J/cm², the suppression of DNA synthesis was accompanied by cell nonviability. The collagen production, when measured immediately following the treatment with 1.7×10^3 J/cm², was markedly reduced, and similar effects were observed with higher energy levels. However, when the cells were tested for collagen production at 20 hours following laser treatment, there was a significant decrease in collagen production at energy levels as low as 1.1×10^3 J/cm², a dose that did not affect DNA synthesis or cell viability. Thus, the results indicate that the Nd:YAG laser can selectively suppress collagen production without affecting cell proliferation. These observations suggest that laser treatment could potentially be used to reduce collagen deposition in conditions such as keloids and hypertrophic scars.

PMID: 6688937 [PubMed - indexed for MEDLINE]

Lasers Surg Med. 1984;4(3):291-5.

[Related Articles](#), [Links](#)

Laser treatment of keloids: a clinical trial and an in vitro study with Nd:YAG laser.

Abergel RP, Dwyer RM, Meeker CA, Lask G, Kelly AP, Uitto J.

Biochemical studies utilizing keloid fibroblast cultures revealed that Nd:YAG laser selectively suppressed collagen production by these cells. Based on these in vitro observations, eight patients with keloids were treated with Nd:YAG laser in a nondestructive manner. Results, with a 3-year follow-up, indicated flattening and softening of the lesions. Thus, the results suggest that Nd:YAG laser is an effective treatment modality for keloids, and its mechanism may involve bioinhibition of fibroblast functions.

Publication Types:

- Clinical Trial

PMID: 6390045 [PubMed - indexed for MEDLINE]

Lasers Surg Med. 2002;31(4):263-7.

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Effect of low-power laser irradiation on cell growth and procollagen synthesis of cultured fibroblasts.

Pereira AN, Eduardo Cde P, Matson E, Marques MM.

Department of Stomatology, School of Dentistry, University of Sao Paulo-SP,
Brazil 05508-900.

BACKGROUND AND OBJECTIVES: In dentistry, low-power lasers have been used in the treatment of dentin hypersensitivity, gingivitis, periodontitis, and different forms of oral ulcers. This in vitro study focuses on the biostimulation of NIH-3T3 fibroblasts by a low-power Ga-As-pulsed laser. **STUDY DESIGN/MATERIALS AND METHODS:** We have studied cell growth and procollagen synthesis of cultured fibroblasts submitted to low-power laser irradiation with energy densities varying from 3 to 5 J/cm(2) over a period of 1-6 days. The light source was a 120 mW Ga-As diode laser ($\lambda = 904$ nm). Growth curves and procollagen immunoprecipitation were obtained. **RESULTS:** Irradiation of 3 and 4 J/cm(2) increased the cell numbers about threefold to sixfold comparing to control cultures. However, this effect was restricted to a small range of energy densities since 5 J/cm(2) had no effect on cell growth. The energy density of 3 J/cm(2) remarkably increased cell growth, with no effect on procollagen synthesis, as demonstrated by the immunoprecipitation analysis. **CONCLUSIONS:** Our results showed that a particular laser irradiation stimulates fibroblast proliferation, without impairing procollagen synthesis. Copyright 2002 Wiley-Liss, Inc.

PMID: 12355572 [PubMed - indexed for MEDLINE]

Low level 809-nm diode laser-induced in vitro stimulation of the proliferation of human gingival fibroblasts.

Kreisler M, Christoffers AB, Al-Haj H, Willershausen B, d'Hoedt B.

Department of Oral Surgery, Johannes Gutenberg-University, Mainz,
Augustusplatz 2, 55131 Mainz, Germany. matthiaskreisler@web.de

BACKGROUND AND OBJECTIVE: The authors investigated the effects of low level laser irradiation on the proliferation rate of human gingival fibroblasts (HGF) in vitro. **STUDY DESIGN/MATERIALS AND METHODS:** HGF were obtained from gingival connective tissue explants and cultured under standard conditions. 110 cell cultures in their logarithmic growth phase were spread on 96-well tissue culture plates and were irradiated at energy fluences of 1.96-7.84 J/cm². Another 110 cultures served as control. An 809-nm semiconductor laser operated at a power output of 10 mW in the cw-mode was used. The time of exposure varied between 75 and 300 seconds. Laser treatment was performed alternatively once, twice, and three times at a 24-hour interval. After lasing, incubation was continued for 24 hours. The proliferation rate was determined by means of fluorescence activity of a redox indicator added to the cell culture. Proliferation was determined 24, 48, and 72 hours after irradiation and expressed in relative fluorescence units (RFU). **RESULTS:** The irradiated cells revealed a considerably higher proliferation activity. The differences were highly significant 24 hour after irradiation (Mann-Whitney U-test, $P < 0.05$) but decreased in an energy-dependent manner after 48 and 72 hour after irradiation. **CONCLUSIONS:** A cellular effect of the soft laser irradiation on HGF is evident. Its duration, however, seems to be limited. These findings might be clinically relevant, indicating that repeated treatments are necessary to achieve a positive laser effect in clinical applications. Copyright 2002 Wiley-Liss, Inc.

PMID: 12116329 [PubMed - indexed for MEDLINE]

Comparison of the low level laser therapy effects on cultured human gingival fibroblasts proliferation using different irradiance and same fluence.

Almeida-Lopes L, Rigau J, Zangaro RA, Guidugli-Neto J, Jaeger MM.

Institute for Research and Development, Universidade Vale do Paraiba-SJC, Brazil. lal@apcd.org.br

BACKGROUND AND OBJECTIVE: The low level laser therapy (LLLT) has been used in Dentistry to improve wound healing. In order to analyse the effect of LLLT on the in vitro proliferation of gingival fibroblasts we developed a primary culture of human gingival fibroblasts. **STUDY DESIGN/MATERIALS AND METHODS:** The cell line named LMF was grown in Dulbecco's Modified Eagle's medium (DME) with either 5% (nutritional deficit) or 10% fetal bovine serum (fbs). Laser irradiation was carried out with diode lasers with the following wavelengths: 670 nm (L1), 780 nm (L2), 692 nm (L3), and 786 nm (L4). The fluence was fixed in 2 J/cm(2). For growth analysis, control (not irradiated) and treated cultures (irradiated) were plated in 60 mm diameter culture dishes for 12 h before the irradiation. **RESULTS:** We found that cells cultured in nutritional deficit condition grown in medium supplemented by only 5% fbs presented a cell proliferation rate significantly smaller than cell grown in ideal culture conditions (10% fbs). However, when irradiated, cells in nutritional deficit presented cell growth similar or higher than that of control cells grown in ideal culture conditions. Using the same fluence, the infrared laser induced a higher cell proliferation than visible laser when the power outputs were different. However, lasers of equal power output presented similar effect on cell growth independently of their wavelengths. **CONCLUSIONS:** The LLLT acts by improving the in vitro fibroblast proliferation and a smaller laser exposure time results in higher proliferation. Copyright 2001 Wiley-Liss, Inc.

PMID: 11553908 [PubMed - indexed for MEDLINE]

Lasers Surg Med. 1994;14(4):347-54.

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Effect of low level diode laser irradiation of human oral mucosa fibroblasts in vitro.

Loevschall H, Arenholt-Bindslev D.

Department of Oral Pathology, Medicine and Toxicology, Royal Dental College, Faculty of Health Sciences, University of Aarhus, Denmark.

The effects of low level laser (LLL) irradiation on the proliferation of human buccal fibroblasts were studied. A standardized LLL set-up was developed (812 nm, 4.5 +/- 0.5 mW/cm²). Cultures in petridishes were divided into eight groups (1 group served as control). On day 6 after seeding, routine growth medium was replaced with PBS for 1/2 hour. At the beginning of this period, LLL irradiation was performed for 0, 1, 3, 10, 32, 100, 316, or 1,000 seconds, respectively--corresponding to the radiant exposures 0, 4.5, 13.5, 45, 144, 450, 1,422, 4,500 mJ/cm². Subsequently the cells received 3H-dT in fresh medium for 16 hours DNA-incorporation. Scintillations from tritium and total protein concentration per culture dish were determined. The individual 3H-cpm/protein-concentration ratios were calculated in % of control. Three experiments were performed (N = 151). Following LLL exposure the 3H-cpm/protein ratio was increased with maximum cpm/protein ratio (132.5% +/- 10.6% SEM) in the group receiving 450 mJ/cm² (P < 0.03 nonparametric Kruskal Wallis one-way ANOVA-test). This study demonstrated an increased incorporation on tritiated thymidine in cultured human oral fibroblasts following LLL exposure and suggests that LLL irradiation can induce increased DNA synthesis.

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